c) a glycosyltransferase comprising the amino acid sequence SEQ ID NO:4, or a functionally active fragment thereof;

d) a glycosyltransferase comprising the amino acid sequence SEQ ID NO:5, or a functionally active fragment thereof;

e) a glycosyltransferase comprising the amino acid sequence SEQ ID NO:6, or a functionally active fragment thereof.

REMARKS

Claims 34-49 are pending in the current application. By way of the present Amendment, claims 34-39 are amended.

Claims 34-49 are pending in the application. Claims 1-26 and 28-31 were canceled by way of the first Preliminary Amendment, filed on December 3, 2001, with the instant application. Claims 27, 32, and 33 were cancelled and claims 34-49 were added by way of the second Preliminary Amendment, filed on April 12, 2002. Therefore, claims 34-49 are presently under consideration.

Claims 34-39 have been amended herein to more particularly point out and distinctly claim the subject matter which Applicants regard as their invention. Support for these amendments is found throughout the specification as filed as more fully set forth below. Thus, no new matter has been added by way of these amendments.

Amendments to the Application to Correct Typographical Errors

Applicant has amended the specification herein merely to correct inadvertent typographical and editing errors, and to correctly identify the Sequence Identification numbers.

As indicated in the Preliminary Amendment filed along with the present application on December 3, 2001, the present application is related to U.S. application no. 08/878,360, filed June 18, 1997, now U.S. Patent 5,945,322, U.S. application no. 08/683,426, filed July 18, 1996, now U.S. Patent 5,705,367, and U.S. application no. 08/312,387, filed September 6, 1994, now U.S. Patent 5,545,553. The amendments to the specification described in detail herein below correspond to amendments made in each of

the above-referenced related patent applications for the purpose of correcting the same inadvertent typographical and editing errors, and accordingly, the present amendments merely serve to ensure that the present application is in conformance with the applications to which it claims priority.

Sequence Identification Amendments

Applicant has amended the specification herein merely to correct inadvertent typographical and editing errors regarding the Sequence Identification numbers, and to correctly identify the Sequence Identification numbers.

SEQ ID NOS:11 and 12 refer to LgtA and LgtD polypeptides, respectively, as set forth in Figure 3 as originally filed. SEQ ID NOS:11 and 12 *correspond* to LgtA and LgtD polypeptides as set forth in SEQ ID NOS:3 and 5, respectively, with the only exception being that the first amino acid is leucine rather than methionine. For example, though the lgtA TTG codon in the corresponding position in SEQ ID NO:1 ordinarily encodes leucine (SEQ ID NO:1 nucleotides 445-447), in *Neisseria*, the same TTG codon encodes methionine. Thus, the sequences of SEQ ID NOS:3 and 5 are correct. However, the amino acid sequences for LgtA and LgtD polypeptides in Figure 3 (SEQ ID NOS:11 and 12, respectively) include leucine as the first amino acid, such as would be found if the protein were expressed in a non-*Neisseria* expression system, such as those expression systems described in the specification at page 25, lines 1-12.

Amendments to the Drawings

Figure 2 has been amended to delete Figure 2B, which corresponds to the sequence listing submitted with the present application. Applicants submit herewith seven (7) sheets of cancelled drawings corresponding to Figure 2B, and eleven (11) sheets of amended formal drawings. The drawings have merely been amended to renumber the sheets of drawings as a result of the cancellation of Figure 2B, and accordingly, no new matter has been added by way of this amendment.

Amendments to Claims 34 and 39

Claims 34 and 39 have been amended to correctly refer to the Sequence Identification numbers, which have been amended in the above-described amendments to the specification to correct inadvertent typographical and editing errors.

The present application claims, in part, an invention comprising five (5) novel glycosyltransferases from *Neisseria*. Specifically, the present application claims lgtA, lgtB, lgtC, lgtD, and lgtE polypeptides.

Accordingly, the five glycosyltransferases of the present invention are the object of claims 34-39, as indicated by the recitation of the identifying term, "glycosyltransferase," followed by the recitation of a SEQ ID number for each one of the LgtA, LgtB, LgtC, LgtD, and LgtE glycosyltransferases. SEQ ID NO:3 correctly refers to lgtA, SEQ ID NO:4 correctly refers to lgtC, SEQ ID NO:5 correctly refers to lgtD, and SEQ ID NO:6 correctly refers to lgtE. However one of the five glycosyltransferases of the present invention, LgtB, was incorrectly referred to using the term "SEQ ID NO:2" in claims 34 and 39.

SEQ ID NO:2, as set forth in the Sequence Listing in the present application, is a polynucleotide sequence encoding a glycyl tRNA synthetase beta chain, and is not a *Neisseria* glycosyltransferase. SEQ ID NO:8 correctly refers to LgtB, a glycosyltransferase of the present invention. Therefore, the above-described amendments to the application serve in part to make proper reference to the LgtB glycosyltransferase (SEQ ID NO:8) of the present invention, and to eliminate any incorrect reference to glycyl tRNA synthetase (SEQ ID NO:2), which is not claimed as part of the present invention.

More particularly, claims 34 and 39 have been amended in part to correctly refer to the Sequence Identification numbers, which numbers have been amended by way of the above-described amendments to the specification to correct inadvertent typographical and editing errors. Specifically, the incorrect recitation of "SEQ ID NO:2" in claims 34 and 39 has been changed so that the claims now correctly recite "SEQ ID NO:8." Prior to the instant amendment, claims 34 and 39 incorrectly recited, "...glycosyltransferase comprising the amino acid sequence SEQ ID NO:2, or a functionally active fragment thereof." Amended claims 34 and 39 now properly recite

"SEQ ID NO:8," which is the LgtB glycosyltransferase of the present invention.

All of the above-detailed amendments to the application have merely been made to correct informalities with the present application. Accordingly, Applicant respectfully submits that no new matter has been added by way of the amendments. Support for all of the above-described amendments to the application can be found in the as-filed application, and specifically, in the Sequence Listing.

The Sequence Listing filed in connection with the present application sets forth that SEQ ID NO:3 is LgtA, SEQ ID NO:4 is LgtC, SEQ ID NO:5 is LgtD, SEQ ID NO:6 is LgtE, and that SEQ ID NO:8 is LgtB. Specifically, SEQ ID NO:1 sets forth a *Neisseria* polynucleotide sequence which also displays the amino acid translation of the coding regions of SEQ ID NO:1. The "Information for SEQ ID NO:1" set forth on pages 1 and 2 of the Sequence Listing of the present application defines SEQ ID NO:1 as encoding glycyl tRNA synthetase beta chain from nucleotides 1-381 of SEQ ID NO:1, encoding LgtA from nucleotides 445-1491 of SEQ ID NO:1, encoding LgtC from nucleotides 2342-3262 of SEQ ID NO:1, encoding LgtD from nucleotides 3322-4335 of SEQ ID NO:1, and encoding LgtE from nucleotides 4354-5196 of SEQ ID NO:1.

Each of the five proteins encoded by SEQ ID NO:1 – four glycosyltransferases and one glycyl tRNA synthetase – are set forth in SEQ ID NOS:2-6 in the Sequence Listing. SEQ ID NO:2 sets forth the amino acid sequence for the glycyl tRNA synthetase beta chain encoded by nucleotides 1-381 of SEQ ID NO:1, SEQ ID NO:3 sets forth the amino acid sequence for the LgtA glycosyltransferase encoded by nucleotides 445-1491 of SEQ ID NO:1, SEQ ID NO:4 sets forth the amino acid sequence for the LgtC glycosyltransferase encoded by nucleotides 2342-3262 of SEQ ID NO:1, SEQ ID NO:5 sets forth the amino acid sequence for the LgtD glycosyltransferase encoded by nucleotides 3322-4335 of SEQ ID NO:1, and SEQ ID NO:6 sets forth the amino acid sequence for the LgtE glycosyltransferase encoded by nucleotides 4354-5196 of SEQ ID NO:1.

Also in the Sequence Listing filed in connection with the present application, SEQ ID NO:7 sets forth a *Neisseria* polynucleotide sequence which also displays the amino acid translation of the coding regions of SEQ ID NO:7. The "Information for SEQ ID NO:7" set forth on page 14 of the Sequence Listing of the

present application defines SEQ ID NO:7 as encoding LgtB from nucleotides 1491-2330 of SEQ ID NO:7. SEQ ID NO:8 sets forth the amino acid sequence for the LgtB glycosyltransferase encoded by nucleotides 1491-2330 of SEQ ID NO:7.

Rejection under 35 U.S.C. § 101

The Examiner has rejected claims 34-49 under 35 U.S.C. § 101 because, in the Examiner's view, the claimed invention is non-patentable, as the claimed invention is directed to "a glycosyltransferase," which reads on "a product of nature." Applicants, while not necessarily agreeing with the Examiner's reasoning, in a good faith effort to expedite prosecution of this application, have amended claims 34-39, from which claims 40-49 depend, to recite "an isolated glycosyltransferase" instead of "a glycosyltransferase." This amendment introduces no new subject matter and is supported throughout the specification and claims as filed (e.g., specification at page 13 lines 5-10, and at page 19 line 21 through page 24 line 22). Applicants respectfully submit that claims 34-49, as amended, are indeed patentable and accordingly request withdrawal of the rejection.

Rejection under 35 U.S.C. § 112, second paragraph

The Examiner has rejected claims 34-49 under 35 U.S.C. § 112, second paragraph, as allegedly being indefinite for failing to point out and distinctly claim the subject matter that the applicant regards as the invention. Specifically, it is the Examiner's view that Applicant's do not clearly define the "functional activity" with respect to the claimed invention.

Applicants respectfully contend that "functional activity" of a glycosyltransferase of the invention is clearly defined throughout the specification as filed. For example, lines 10-15 on page 22 of the specification define a "functionally active fragment" as a glycosyltransferase fragment that is capable of mediating transfer of a sugar to an acceptor molecule. The definition is further clarified by contrast with functionally inactive fragments at lines 16-20 on page 22 of the specification, which fragments are not required for functional activity as described above.

Additionally, specific examples of the activity of functionally active

glycosyltransferases are given at page 7, lines 6-10. Further, lines 1-5 on page 21 describe how one skilled in the art can identify a functionally active fragment of a glycosyltransferase of the present invention, in particular by the ability of such a fragment to mediate transfer of a sugar to an acceptor molecule. Accordingly, Applicants submit that the term "functionally active fragment thereof" is not indefinite, and request that the rejection be reconsidered and withdrawn.

Rejection under 35 U.S.C. § 103(a)

The Examiner has rejected claim 39 under 35 U.S.C. § 103(a) as being unpatentable over U.S. Patent No. 5,180,674 of Roth ("the '674 patent"). Specifically, it is the Examiner's view that the '674 patent teaches a method of immobilization of a glycosyltransferase to a solid support, and that one of skill in the art would have been sufficiently motivated to immobilize specific glycosyltransferases of the present invention in light of the '674 patent.

Prior to Applicant's rebuttal of the Examiner's rejection, Applicant wishes to point out that the SEQ ID numbers referred to hereinbelow are the correct, amended SEQ ID numbers for the five glycosyltransferases of the present invention, as described in greater detail above. Specifically, SEQ ID NOS:3-6 and 8 refer to glycosyltransferases lgtA, lgtC, lgtD, lgtE, and lgtB, respectively.

Applicants respectfully submit that the embodiment of the present invention set forth in claim 39 would **not** have been obvious to one of skill in the art, and that the '674 patent does not render claim 39 *prima facie* obvious under 35 U.S.C. § 103(a), for the following reasons.

The three-prong test which must be met for a reference or a combination of references to establish a *prima facie* case of obviousness has not been satisfied in the instant matter. The MPEP states, in relevant part:

> To establish a *prima facie* case of obviousness, three basic criteria must be met. First, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. Second, there must be a reasonable expectation of success. Finally, the prior art reference (or references

when combined) must teach or suggest all of the claim limitations. MPEP § 2142.

Not all of these criteria have been met here.

Assuming, *arguendo*, that one of skill in the art would have been motivated to attempt to immobilize a glycosyltransferase on a solid support, and with a reasonable expectation of success, the overriding fact remains that all of SEQ ID NOS:3-6 and 8, glycosyltransferases lgtA, lgtC, lgtD, lgtE, and lgtB, respectively, were **unknown before the time of filing of the present patent application**. None of the prior art cited by the examiner - neither the cited prior art reference (the '674 patent) nor the knowledge of one skilled in the relevant art prior to the time of filing of the present application – teaches or suggests **all** of the claim limitations. Particularly, because *Neisseria* glycosyltransferases lgtA, lgtC, lgtD, lgtE, and lgtB (SEQ ID NOS:3-6 and 8, respectively) were **unknown** before the time of filing of the present patent application, such limitations of instant claim 39 would not have been available to one of skill in the art to rely upon for teaching or suggesting the present invention in conjunction with the '674 patent. Thus, the combination of the prior art cited cannot render the present invention *prima facie* obvious under 35 U.S.C. 103(a).

In fact, the Examiner's rejection is based on improper hindsight reasoning. The sequences represented by SEQ ID NOs:3-6 and 8 in the present application are novel amino acid sequences disclosed for the first time in the present invention. Consequently, one of skill in the art would **only** be motivated to immobilize the glycosyltransferases represented by SEQ ID NOs:3-6 and 8 when armed with the disclosure of the present application. See MPEP 2145(X). Accordingly, Applicants respectfully submit that the rejection is improper and should be withdrawn.

Double Patenting Rejection

The Examiner has rejected claims 35-39, 41-44, and 46-49 under the judicially created doctrine of obviousness-type double patenting. Specifically, it is the Examiner's view that the above-mentioned claims are not patentably distinct from claims 1-5, 7-10, 12-16 and 18 of U.S. Patent No. 5,798,233 of Gotschlich ("the '233 patent").

Applicant understands that a timely filed Terminal Disclaimer in compliance with 37 CFR § 1.321(c) may be used to overcome such a non-statutory type of double patenting rejection. Accordingly, Applicant is filing a Terminal Disclaimer herewith to overcome the double patenting rejection of claims 35-39, 41-44, and 46-49.

Sequence Compliance

In the Office Action, the Examiner requested compliance with sequence rules. Specifically, the Examiner noted that the sequences depicted in Figures 3, 4 and 5 are not identified with a SEQ ID number.

Applicant has amended the Brief Descriptions of Figures 3, 4 and 5 to formally identify each of the amino acid sequences in Figures 3, 4 and 5 with a SEQ ID number. Specifically, Applicant has inserted the corresponding SEQ ID number following each recitation of the name of the amino acid sequence in the Brief Descriptions of Figures 3, 4 and 5. More particularly, Figure 3 illustrates an amino acid sequence comparison of LgtA and LgtD, SEQ ID NOS:11 and 12, respectively, Figure 4 illustrates an amino acid sequence comparison of LgtB and LgtE, SEQ ID NOS:8 and 6, respectively, and Figure 5 illustrates an amino acid sequence comparison between LgtC and rfaI, SEQ ID NOS:4 and 13, respectively.

Further, Applicant has submitted an amended copy of the Sequence Listing, which amended copy is identical to the Sequence Listing originally filed with the present application except for the addition of SEQ ID NO:13, which is the rfaI sequence found in Figures 5A and 5B. Applicant respectfully submits that the amino acid sequence of SEQ ID NO:13 is identical to the rfaI amino acid sequence set forth in Figures 5A and 5B. Accordingly, no new matter has been added to the application by way of this amendment.

Summary

The amendments made herein are supported in the as-filed specification, and as such, no new matter has been added by way of the present amendment. Applicant respectfully submits that each and every rejection or objection set forth by the Examiner has either been overcome or is now inapplicable, and that the instant application is in full condition for allowance. Favorable examination of the claims on the merits is respectfully requested.

Respectfully submitted,
EMIL C. GOTSCHLICH

(Date)

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Enclosures (Sequence Listing, CRF of Sequence Listing, Statement to Support Sequence Listing, Drawing Transmittal Sheet, Seven Sheets of Cancelled Drawings, Eleven Sheets of Amended Formal Drawings, Terminal Disclaimer, Terminal Disclaimer Transmittal Sheet)

Marked-up version of the specification to show changes made

Please delete the paragraph from page 4 line 15 to page 5 line 2, and insert the following paragraph in place thereof:

-- Little information on the genetics of LOS synthesis ~~of~~ in Neisseria is available. A major advance has been the creation (Dudas and Apicella, 1988, Infect. Immun. 56:499) and biochemical characterization (John et al., 1991, J. Biol. Chem. 266:19303) of five pyocin mutants of gonococcal strain 1291, dubbed 1291a-e. Immunological and biochemical data have shown that 1291a, 1291c, 1291d and 1291e produce LOS with sequential shortening of the lacto-N-neotetraose chain, with mutant 1291e lacking the glucose substitution on the heptose. Mutant 1291b synthesizes the alternative LOS structure Galα1 $\rightarrow$ 4Galβ1 $\rightarrow$ 4Glc (see Figure 1). Only the genetic basis of the 1291e mutant is now defined. It is a mutation of phosphoglucomutase (pgm), which precludes the synthesis of UDP-glucose, and hence the addition of the first residue of the lacto-N-neotetraose unit (Zhou et al., 1994, J. Biol. Chem. 269:11162; Sandlin and Stein, 1994, J. Bacteriol. 176:2930). It also has been shown that gale mutants of meningococcus or gonococcus produce truncated LOS in keeping with the inability to synthesize UDP-galactose (Robertson et al., 1993, Molec. Microbiol. 8:891; Jennings et al., 1993, Molec. Microbiol. 10:361). --

Please delete the paragraph at lines 13-23 on page 6 and insert the following paragraph in place thereof:

-- The present invention is directed to nucleic acids encoding glycosyltransferases, the proteins encoded thereby, and to methods for synthesizing oligosaccharides using the glycosyltransferases of the invention. Accordingly, in one aspect, the invention is directed to a purified nucleic acid that is hybridizable under moderately stringent conditions to a nucleic acid corresponding to the LOS locus of Neisseria, e.g., a nucleic acid having a nucleotide sequence corresponding to or complementary to the nucleotide sequence shown in ~~Figure 2~~ (SEQ ID NO:1). Preferably, the nucleic acid of the invention is hybridizable to a portion of the coding sequence for a gene of the LOS locus, i.e., a portion of the nucleotide sequence shown in

~~Figure 2~~ (SEQ ID NO:1) that encodes a functionally active glycosyltransferase. --

Please delete the paragraph on page 6 line 24 to page 7 line 5 and insert the following paragraph in place thereof:

-- In specific embodiments, the invention relates to a nucleic acid that has a nucleotide sequence corresponding to or complementary to a portion of the nucleotide sequence shown in ~~Figure 2~~ (SEQ ID NO:1) that encodes a functionally active glycosyltransferase. In a further aspect, the nucleic acid encodes a functionally active glycosyltransferase. In a specific embodiment, the invention is directed to a nucleic acid that has a nucleotide sequence corresponding to or complementary to the nucleotide sequence shown in ~~Figure 2~~ (SEQ ID NO: 1). --

Please delete the paragraph at lines 15-17 on page 7 and insert the following paragraph in place thereof:

-- In specific embodiments, exemplified herein, the nucleic acid encodes a glycosyltransferase having an amino acid sequence of ~~SEQ ID NO:2;~~ SEQ ID NO:3 or SEQ ID NO:11, SEQ ID NO:4, SEQ ID NO:5~~,~~ or SEQ ID NO:12, SEQ ID NO:6, or SEQ ID NO:8. --

Please delete the paragraph at lines 1-13 on page 8 and insert the following paragraph in place thereof:

-- In a primary aspect, the invention is directed to glycosyltransferase having an amino acid sequence of ~~SEQ ID NO:2,~~ SEQ ID NO:3 or SEQ ID NO:11, SEQ ID NO:4, SEQ ID NO:5~~, or~~ or SEQ ID NO:12, SEQ ID NO:6, or SEQ ID NO:8 or a functionally active fragment thereof. The invention further contemplates a composition comprising a glycosyltransferase conjugated to a solid phase support, wherein the glycosyltransferase is selected from the group consisting of a glycosyltransferase having an amino acid sequence of ~~SEQ ID NO:2~~ SEQ ID NO:3 or SEQ ID NO:11, or a functionally active fragment thereof; a glycosyltransferase having an amino acid sequence of SEQ ID ~~NO:3~~ NO:8, or a functionally active fragment thereof; a glycosyltransferase having an amino acid sequence of SEQ ID NO:4, or a functionally

active fragment thereof; and a glycosyltransferase having an amino acid sequence of SEQ ID NO:5 or SEQ ID NO:12, or a functionally active fragment thereof; and a glycosyltransferase having an amino acid sequence of SEQ ID NO:6, or a functionally active fragment thereof. --

Please delete the paragraph from page 8 line 14 to page 9 line 3, and insert the following paragraph in place thereof:

-- Having provided novel glycosyltransferases, and genes encoding the same, the invention accordingly further provides methods for preparing oligosaccharides, *e.g.*, two or more saccharides. In specific embodiments, the invention relates to a method for adding GalNAc or GlcNAcβ1→3 to Gal, comprising contacting a reaction mixture comprising an activated GalNAc or GlcNAc to an acceptor moiety comprising a Gal residue in the presence of the glycosyltransferase having an amino acid sequence of SEQ ID ~~NO:2~~ NO:3 or SEQ ID NO:11; a method for adding Galβ1→4 to GlcNAc or Glc, comprising contacting a reaction mixture comprising an activated Gal to an acceptor moiety comprising a GlcNAc or Glc residue in the presence of the glycosyltransferase having an amino acid sequence of SEQ ID ~~NO:3~~ NO:8; a method for adding Gal α1→4 to Gal, comprising contacting a reaction mixture comprising an activated Gal to an acceptor moiety comprising a Gal residue in the presence of the glycosyltransferase having an amino acid sequence of SEQ ID NO:4; a method for adding GalNAc or GlcNAc β1→3 to Gal, comprising contacting a reaction mixture comprising an activated GalNAc or GlcNAc to an acceptor moiety comprising a Gal residue in the presence of the glycosyltransferase having an amino acid sequence of SEQ ID NO:5 or SEQ ID NO:12; and a method for adding Gal β1→4 to GlcNAc or Glc, comprising contacting a reaction mixture comprising an activated Gal to an acceptor moiety comprising a GlcNAc or Glc residue in the presence of the glycosyltransferase having an amino acid sequence of SEQ ID NO:6. --

Please delete the paragraph from lines 3-12 on page 11, and insert the following paragraph in place thereof:

-- Figure 2: ~~(A)~~ Genetic map of the LOS locus based on the DNA sequence. Sequence information bp 1-2725 was obtained from plasmid pPstCla, bp 2725-5859 from plasmid p3400 (see materials and methods). IS refers to an area of the sequence that has homology to a previously reported neisserial insertion sequence IS1106 (Knight et al., 1992, Molec. Microbiol. 6:1565). The positions of the reading frames of *lgtA-E* are indicated. Three tracts of poly-G were found in *lgtA* (17 bp), *lgtC* (10 bp) and *lgtD* (11 bp) and are indicated by vertical black bars. ~~(B) Amino acid sequences of LgtA (SEQ ID NO:2), LgtB (SEQ ID NO:3), LgtC (SEQ ID NO:4), LgtD (SEQ ID NO:5), and LgtE (SEQ ID NO:6), and the nucleotide sequence of the lgt locus (SEQ ID NO:1).~~ --

Please delete the paragraph from lines 13-18 on page 11, and insert the following paragraph in place thereof:

-- Figure 3 (A and B): Homology of the protein products of *lgtA* (SEQ ID NO:11) and *lgtD* (SEQ ID NO:12). The primary structure of two proteins is very similar, particularly in the first half of the sequences. The glycine residues starting at position 86 reflect the coding of the poly-G regions in the respective genes. The Bestfit program of the GCG package was used and the symbols |, :, . represent degrees of similarity based on the Dayhoff PAM-250 matrix. --

Please delete the paragraph from lines 19-23 on page 11, and insert the following paragraph in place thereof:

-- Figure 4 (A and B): Homology of the protein products of *lgtB* (SEQ ID NO:8) and *lgtE* (SEQ ID NO:6). The primary 20 structure of two proteins is very similar, particularly in the first half of the sequences. These sequences also have significant homology to *lex-1* (Cope et al., i99i, Molec. Microbiol. 5:i113) or *lic2A* (High et al., i993, Molec. Microbiol. 9:1275) genes of *Haemophilus influenzae*. For meaning of symbols see Figure 3. --

Please delete the paragraph from page 11 line 24 to page 12 line 2, and insert the following paragraph in place thereof:

-- Figure 5 (A and B): Homology of the protein products of *rfaI* (SEQ ID NO:13) and *lgtC* (SEQ ID NO:4). The *E. coli rfaI* and *rfaJ* genes are very closely related. They serve as glucosyl transferases of two glucose residues in the LPS core region (Pradel et al., 1992, J. Bacteriol. 174:4736). The glycines at position 54-56 in *lgtC* are encoded by the poly-G tract. For meaning of symbols see Figure 3. --

Please delete the paragraph from lines 11-29 on page 30, and insert the following paragraph in place thereof:

-- Accordingly, a method for preparing an oligosaccharide having the structure GalNAcβ1→3Galβ1→4GlcNAcβ1→3Galβ1→4Glc *(i.e.,* ganglioside) comprises sequentially performing the steps of:

d. contacting a reaction mixture comprising an activated Gal to an acceptor moiety comprising a Glc residue in the presence of a glycosyltransferase having an amino acid sequence of SEQ ID NO: 6, or a functionally active fragment thereof;

e. contacting a reaction mixture comprising an activated GlcNAc to the acceptor moiety comprising a Galβ1→4Glc residue in the presence of a glycosyltransferase having an amino acid sequence of SEQ ID ~~NO:2~~ NO:3 or SEQ ID NO:11, or a functionally active fragment thereof;

f. contacting a reaction mixture comprising an activated Gal to the acceptor moiety comprising a GlcNAcβ1→3Galβ1→4Glc residue in the presence of a glycosyltransferase having an amino acid of SEQ ID ~~NO:3~~ NO:8; and

g. contacting a reaction mixture comprising an activated GalNAc to the acceptor moiety comprising a Galβ1→4GlcNAcβ1→3Galβ1→4Glc residue in the presence of a glycosyltransferase having an amino acid sequence of SEQ ID NO:5 or SEQ ID NO:12, or a functionally active fragment thereof. --

Please delete the paragraph from lines 1-14 on page 31, and insert the following paragraph in place thereof:

-- Similarly, a method for preparing an oligosaccharide having the structure Galβ1→4GlcNAcβ1→3Galβ1→4Glc *(i.e.,* lacto-N-neotetraose) comprises sequentially performing the steps of:

h. contacting a reaction mixture comprising an activated Gal to an acceptor moiety comprising a Glc residue in the presence of a glycosyltransferase having an amino acid sequence of SEQ ID NO: 6, or a functionally active fragment thereof;

i. contacting a reaction mixture comprising an activated GlcNAc to the acceptor moiety comprising a Galβ1→4Glc residue in the presence of a glycosyltransferase having an amino acid sequence of SEQ ID ~~NO:2~~ NO:3 or SEQ ID NO:11, or a functionally active fragment thereof; and

j. contacting a reaction mixture comprising an activated Gal to the acceptor moiety comprising a GlcNAcβ1→3Galβ1→4Glc residue in the presence of a glycosyltransferase having an amino acid of SEQ ID ~~NO:3~~ NO:8. --

Please delete the paragraph from lines 10-26 on page 38, and insert the following paragraph in place thereof:

-- A gene bank of *Neisseria gonorrhoeae* strain F62 genomic DNA was constructed by ligating ca 20 kb fragments obtained by incomplete digestion with *Sau3A* into *Bam*HI/*Eco*RI digested λ2001 (Karn et al., 1984, Gene 32:217). The phage library was screened by hybridization with ~~random-prime-labeled~~random-primer-labeled plasmid pR10PI, and 5 clones were isolated by plaque purification. The phage from these clones were purified by sedimentation followed by flotation on CsCl (Davis et al., 1980, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), and the DNA was isolated. From one of these clones, two *Cla*I fragments of 4.9 and 3.4 kb were isolated by gel electrophoresis and recovery with Geneclean II (BIO 101 Inc., La Jolla, CA). These were ligated into *Cla*I cut pBluescript II SK- from Stratagene (La Jolla, CA) and called p4900 and p3400 respectively. p4900 contained a *Pst*I site in the insert and was subdivided into two clones containing inserts of 2.1 and 2.8 kb. The clone containing the 2.8 kb insert was called pPstCla. The inserts in p3400 and pPstCla were sequenced by

the chain termination method (Sanger et al., 1977, Proc. Natl. Acad Sd. USA 74:5463) using Sequenase II, (United States Biochemical Co., Cleveland, OH). All of the sequence presented in ~~Figure 2~~SEQ ID NO:1 -was completed in both directions. --

Please delete the paragraph from line 13 page 39 to line 3 page 40, and insert the following paragraph in place thereof:

-- Transformation of piliated *Neisseria gonorrhoeae* strain F62 was performed with plasmids isolated from *E. coil* (Klugman et al., 1989, Infect. Immun. 57:2066) and the transformants selected on GC agar (Swanson, 1978, Infect. Immun. 19:320) containing 2 μg/ml erythromycin. The fidelity of the genomic alteration of each of the gonococcal transformants was verified by sequencing the upstream and downstream junctions of the *ermC'* gene in their genomic DNA using a PCR technique. Two 5' biotinylated primers, GCCGAGAAAACTATTGGTGGA (SEQ. ID.~~NO:7~~ NO:9) and AAAACATGCAGGAATTGACGAT) (SEQ. ID.~~NO:8~~ NO:10), were synthesized; these were based on the *ermC'* sequence near its upstream and its downstream end respectively. The primers were designed such that their 3' ends pointed outward from the *ermC'* gene. Each of these primers was used together with a suitable primer matching the sequence of the LOS locus near the putative insertion. PCR was performed according the instructions supplied with the GeneAmp PCR Reagent Kit from Perkin Elmer (Branchburg, NJ) using 25 cycles. In all instances the expected size product was obtained. The DNA sequence of these products was determined by purifying the PCR product on magnetic streptavidin beads from Dynal, Inc. (Lake Success, NY) and sequencing with the Sequenase II kit according to a protocol provided by Dynal, Inc., based on the method developed by Hultman et al (Hultman et al., 1989, Nucleic Acids Res. 17:4937). The sequences were analyzed by computer programs in the GCG package of Genetics Computer Group, Inc. (Madison, WI). --

Please delete the paragraph from lines 15-29 on page 41, and insert the following paragraph in place thereof:

-- A λ2001 bank of *Neisseria gonorrhoeae* strain F62 DNA was screened by hybridization with pR10PI and 5 clones were isolated. One of these clones, when

digested with either *Cla*I or *Bfa*I and examined by Southern hybridization using pR10PI as the probe, gave rise to a pattern identical to that seen with genomic DNA. The appropriate *Cla*I fragments of this λ2001 clone were isolated and cloned into the *Cla*I site of pBluescript II SK-. The entire sequence of the 3400 *Cla*I fragment was determined. Mapping of the clone containing the 4900 bp *Cla*I fragment indicated that there was a single *Pst*I site in the clone about 2.8 kb from one side, allowing the clone to be divided into two subclones. Partial sequence of the ends of the 2.1 kb subclone indicated that it contained a coding frame homologous to the *E. coil* COOH-terminal portion of the α subunit of glycyl-tRNA synthetase *(glyS)* and the majority of the β subunit of this gene (Webster et al., 1983, J. Biol. Chem. ~~258:10637~~ 258:10637). The predicted length of DNA needed to match the *E. coli* sequence was present; this clone was not examined further. --

Please delete the paragraph from lines 1-9 on page 42, and insert the following paragraph in place thereof:

-- *DNA Sequence of the LOS Locus.* A summary of the features found by sequencing the two clones is illustrated in Figure 2. Following the *glyS* gene ~~Ifound~~were found-five closely spaced open reading frames. The last frame has 46 bp downstream of the termination codon a sequence typical of a rho independent termination signal. Subsequently, there is an area of ca 100 bp that has striking homology to the ISI1106 neisserial insertion sequence (Knight et al., 1992, Molec. Microbiol. 6:1565). Further elucidation of the nature of this locus, presented below, showed the five open reading frames code for LOS glycosyl transferases and hence they have been named *lgtA- lgtrE.* --

Please delete the paragraph from lines 10-29 on page 42, and insert the following paragraph in place thereof:

-- Searches for internal homology within this locus indicates that the DNA coding for the first two genes *(lgtA, lgtB)* is repeated as the fourth and fifth genes *(lgtD, lgtE)* and that interposed is an additional open reading frame, *lgtC.* This is in keeping with the data obtained by Southern hybridization presented above, in which

pR10PI probe containing the *lgtB* and a small portion of the *lgtC* gene hybridized with two *Cla*I fragments, but with only one *Bfa*I fragment (see positions of the *Bfa*I sites in the LOS locus in Figure 2). In more detail, 16 bp following the stop codon of the tRNA synthetase *(glyS)* is the beginning of a stem loop structure followed closely by a consensus ribosome binding site (rbs), and within 6 bp is a TTG believed to be the initiation codon of *lgtA*. 2871 bp downstream from the beginning of the stem loop (closely following the stop codon of *lgtC)* there is an almost perfect repeat of the stem loop structure, the ~~rbs~~rbs, and the TTG initiation codon of ~~*lgtD*~~*lgtD*, with the downstream sequence strongly homologous for about 500 bp. The sequences then diverge to some extent. However, at the beginning of *lgtB* and *lgtE* the homology again becomes nearly perfect for ca 200 bases to then diverge toward the latter part of the orfs. The similarity of the homologous proteins is illustrated in Figures 3 and 4. These comparisons, demonstrate the near-perfect conservation of the primary structure in the N-terminal portions of the molecules with increasing divergence toward the COOH-termini of the proteins. --

Marked-up version of the claims to show changes made

Please amend claims 34-39 without prejudice, as set forth below.

34. (Amended) ~~A~~ An isolated glycosyltransferase comprising the amino acid sequence ~~SEQ ID NO:2~~SEQ ID NO:8, or a functionally active fragment thereof.

35. (Amended) ~~A~~ An isolated glycosyltransferase comprising the amino acid sequence SEQ ID NO:3, or a functionally active fragment thereof.

36. (Amended) ~~A~~ An isolated glycosyltransferase comprising the amino acid sequence SEQ ID NO:4, or a functionally active fragment thereof.

37. (Amended) ~~A~~ An isolated glycosyltransferase comprising the amino acid sequence SEQ ID NO:5, or a functionally active fragment thereof.

38. (Amended) ~~A~~ An isolated glycosyltransferase comprising the amino acid sequence SEQ ID NO:6, or a functionally active fragment thereof.

39. (Amended) A composition comprising ~~a~~ an isolated glycosyltransferase conjugated to a solid phase support, wherein the glycosyltransferase is selected from the group consisting of:

a) a glycosyltransferase comprising the amino acid sequence ~~SEQ ID NO:2~~SEQ ID NO:8, or a functionally active fragment thereof;

b) a glycosyltransferase comprising the amino acid sequence SEQ ID NO:3, or a functionally active fragment thereof;

c) a glycosyltransferase comprising the amino acid sequence SEQ ID NO:4, or a functionally active fragment thereof;

d) a glycosyltransferase comprising the amino acid sequence SEQ ID NO:5, or a functionally active fragment thereof;

e) a glycosyltransferase comprising the amino acid sequence SEQ ID

NO:6, or a functionally active fragment thereof.